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Characterization, Fluxes and Toxicity of Leather Tanning Bath Chemicals in a Large Tanning District Area (IT)

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Abstract The leather tanning industry is perceived as responsible of a significant consume of natural resources and output of wastes such as high concentration of organics, salts and heavy metals such as chromium, both in solid and liquid form as a result. Now as ever the future of tanneries strongly depends on the increase of their awareness that a sustainable industry for the future means embracing a forward-looking philosophy of the leather making process through optimal resource management within the tannery. This study reports the characterization of some chemicals used in a large tanning district area in terms of COD, BOD, aromaticity (UV_{280}) and double bond (UV_{254}) absorbance measurements, toxicity on fresh and saline water as well as terrestrial species, GC-MS scanning. The study provides a consistent set of information on tannin-associated concentration-related trends and suggests novel criteria in defining control quality for evaluating environmental impact of leather tanning industry.

Keywords Tannery chemicals · Synthetic tannins · Green chemistry · GC-MS · Toxicity · *Daphnia magna* · *Artemia salina* · *Selenastrum capricornutum* · *Lepidium sativum* · Xenobiotics

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1 Introduction

The impact of leather tanning industry on the environment is an ongoing and increasing problem. Water consumption varies greatly between tanneries, depending on the processes involved, the raw material used and the manufactured products. However a large amount of freshwater is used to treat leather and many potentially dangerous chemicals such as chromium, synthetic tannins, oils, resins, biocides, detergents, are released (Suresh et al. 2001; Nazer et al. 2006). The concerns related to effluents originated from this industry are mainly due to chronic toxic effects caused by the mixture of many compounds used in the process that can be released to the environment because they even remain after conventional treatment (Meriç et al. 2005; Oral et al. 2007; De Nicola et al. 2007a) or may inhibit nitrification process (Jochimsen and Jekel 1997).

Unlike the extensive literature on chromium toxicity, only a scarce database is available on the environmental impact of the leather tanning processes based on vegetable and synthetic tannins, or syntans (STs) (De Nicola et al. 2007b) or on retanning operations utilizing Synthetic tannins (STs) (Lofrano et al. 2007a). A class of chemicals synthesized for tanning or retanning purposes in the leather tanning industry is collectively termed STs, which are characterized by complex chemical structures because they are composed of an extended set of chemicals such as phenol-, naphthalene-, -formaldehyde and melamine-based syntans, and acrylic resins. The STs are not completely fixed by skins and remain in the effluent. Individual components such as phenols, formaldehyde are persisting conventional chemical and/or biological wastewater treatment processes (Lofrano et al. 2007a). The toxicity of mimosa tannin and phenol-based syntan was tested for toxicity to sea urchin (*Paracentrotus lividus* and *Sphaerechinus granularis*) early development and to marine algal growth (*Dunaliella tertiolecta*) comparatively. Sea urchin embryogenesis was affected by vegetable tannin and syntan water extracts at levels 1 mg/L (De Nicola et al. 2007b). Cell growth bioassays in *D. tertiolecta* exposed to vegetable tannin and syntan water extracts showed non-linear concentration-related toxicity.

The EU passed in July 2003 the directive 2003/53/EC, which will restrict the marketing and use of products and product formulations that contain more than 0.1% of nonyl ethoxyphenol (NPE) or nonylphenol (NP) in Europe. This applies to many industries including the textile and leather industries, except in the case of closed application systems where no release into wastewaters occurs. From January 2005, this Directive comes into force. However, there has not been any particular restriction yet for synthetic tannins with their complex structure composed of many indescribable chemicals except those chemicals containing formaldehyde which have been banned to use in Italy from 2005, although there has been extensive impact assessment and treatment applications for individual chemicals such as cresols, phenols (Tisler and Zagorc-Koncan 1997; Hohreiter and Rigg 2001; Wang et al. 2002).

Lofrano et al. (2007a) surveyed the characteristics of various tanning chemicals (syntans and oils) used in retanning process in the same leather district. The low biodegradability (BOD_5/COD) of the STs-various based was very low (>0.15) (Lofrano et al. 2007a) and the acute toxic effect of a phenol based-ST on *D. magna* at 300 mg/L was attempted to remove using advanced oxidation processes (AOPs) (Fenton and Photo-Fenton processes) (Lofrano et al. 2007b).

This paper aimed to define characteristics and fluxes of retanning chemicals used in a large tanning district, Southern Italy, in terms of BOD_5/COD ratio, UV–VIS absorbance, GC-MS scanning and ecotoxicological endpoints of immobilization (*Daphnia magna* and *Artemia salina*), cell growth inhibition (*Selenastrum capricornutum*) and plant growth index (*Lepidium sativum*) as a combined effect calculated based on inhibition of germination and root length as a new approach. The toxicity tests are useful analytical tools for screening of chemical analysis and as an early warning system to monitor the treatment of WWTPs. The use of single toxicity test or battery of tests is the best approach to evaluate the risk because they are reliable indices of the toxic impact of effluents in the aquatic environment (Hernando et al. 2005). Thus, this multi-approach study is expected to give essential contribution to develop treatment strategies for leather tanning industry as well as risk assessment procedures.

2 Materials and Methods

2.1 Solutions

Based on our previous experience on tannins toxicity, two synthetic tannins (ST1-sulphonated base, ST2-poly-condensed phenols with formaldehyde) and two resins (R1, R2) as being most frequent used in these baths were obtained from the leather tanning industry district, Southern Italy. The commercial code of these compounds investigated are AUS002 (ST1), KT209600-1 (ST2), KT131804-1 (R1) and KT223300-1 (R2) obtained from KEMIA -TAU (Turin, Italy). ST1 and R1 are registered as "contains substances which are considered dangerous for human health by the European Union (Directive 67/548/EC)" whereas ST2 and R2 are introduced as "no toxicity data are available, no information about their fate in the environment" in their safety sheet. Considering the assessed levels of the tannins and resins as given in Table 3, a range from 10 to 1000 mg/L of the above compounds tested for

toxicity and chemical parameters. 1 g/L of stock solutions of each chemical were prepared daily in double distilled water obtained by a Milli-Q system (Millipore, Milford, MA, USA) and kept refrigerated at +4°C during experiments.

2.2 Chemical Analysis

COD equivalent (mg/L) corresponding to 1 g/L solutions of each compound was determined according to Standard Methods (1998). BOD_5 was measured by the differences of manometric pressure (OXI TOP, ISCO, IT) and by seeding activated sludge taken from nitrification process of a leather tanning wastewater treatment plant. The ratio of BOD_5/COD, which indicates the biodegradability of the compounds, was calculated.

Absorbance values at 254 and 280 nm respectively were determined as triplicate using UV–VIS spectrophotometer (Perkin Elmer, Lambda 12 Model), using 1 cm quartz tube in order to have a measure of double bonds and aromatic structure. Spectrophotometric analyses were run in triplicate.

2.3 GC-MS Detection and Identification Procedure

2.3.1 Samples Preparation for LLE Extraction

The sample preparation procedure included addition of sodium sulfate (Merck) (5 g) in 50-mL sample, liquid–liquid extraction for 5 minutes initially with dichloromethane (Merck, for organic trace analysis) (10 ml) and afterwards with n-pentane (Merck, for organic trace analysis) (10 ml). The extracts were combined and, after addition of sodium sulfate (5 g) for drying, were evaporated to dryness in a water bath at 50°C (Lofrano et al. 2007b; Nikolaou et al. 2002).

2.3.2 Derivatization Procedure

To the vials containing the dried residues of the LLE extraction procedure, a volume of 50 μl of bis-(Trimethylsilyl)trifluoroacetamide (BSTFA, CAS # 25561-30-2) plus 50 μl of pyridine was added. The vials were closed and completely mixed for 1 min using a vortex system. The derivatization reaction was performed at 65°C for 20 min. The derivatives were allowed to cool to room temperature and subjected to GC-MS analysis (Gatidou et al. 2007).

2.3.3 GC-MS Analysis

The screening of the ST1, ST2, R1 and R2 was performed by liquid–liquid extraction followed by gas chromatography-mass spectrometry using a Thermo Finnigan model gas chromatograph connected to a TRACE DSQ model MSD, supported from the Excalibur software. The separation of compounds was conducted on a DB-5MS column (30 m× 0.32 mm internal diameter × 0.25 μm film thickness). Helium was used as carrier gas (flow 0.9 ml/min) and the injection technique was splitless (injector temperature was 280°C). The oven temperature program was 80°C for 1 min, 15°C /min to 220°C, 5°C/min to 280°C. The MS transfer line temperature was kept at 280°C, whereas the ion source temperature was 180°C. The MS was operated in SCAN mode. The method used for samples analysis was adopted from a previously published method with minimal modification (Lofrano et al. 2007b).

2.4 Toxicity Tests

2.4.1 Daphnia magna

Newborn daphnids (<24 hours old) were exposed to chemicals solutions at different concentrations for 24 hours according to standard methods (ISO 1996; Guida et al. 2008). Tests were conducted in quadruplicate with a photoperiod of 16 hours light (with 1000 lx) and 8 hours dark, using 5 daphnids in each test beaker with 100 ml effective volume. They were regularly fed with the algae *Selenastrum capricornutum* (300,000 cells/ml) and *Schizosaccharomyces cerevisiae* (200,000 cells/ml). A blank was also run in parallel series. The results were expressed as a percentage of immobilised animals after 24 and 48 h exposure time.

2.4.2 Artemia salina

Artemia cysts (RAC) (AF/N2000) were obtained from the Quality Assurance Research Division, USEPA, Cincinnati, OH, USA and the Laboratory for Biological Research in Aquatic Pollution, University of Ghent, Belgium by the certification of *A. franciscana* in Italy (ECOTOX srl). The cysts were activated in a standard marine solution (35‰ salinity, Ocean®). *A. franciscana* cysts (<48-h old) were exposed to the

same samples with *D. magna*. Both negative control with Ocean® and positive control with $K_2Cr_2O_7$ tests were performed in parallel (Migliore et al. 1997; Guida et al. 2008).

2.4.3 Selenastrum capricornutum

Unicellular freshwater green microalgae from the species *S. capricornutum* were used. Weekly transplanted algae in exponential growth were cultured in a salt solution (ASTM 1986; ISO 2004; Guida et al. 2008) dissolved in ultra pure water. Cultures were kept in Erlenmeyer flasks at the same conditions with *D. magna* and algae were exposed to the same samples with *D. magna*. The toxicity tests were initiated from an algal concentration of 3,000 cells/mL and conducted in nine replicates. Negative and positive control tests were performed in parallel. The endpoint consisted of algal growth, which was measured after 96 h in a Bürker cell counting chamber.

2.4.4 Lepidium sativum

Cress seed (*L. sativum* L.) test is evaluated for the multiplication factor of two end points i) germinated seed number and ii) root length. General literature focused on for each of these endpoints (Fuentes et al. 2006; Moldes et al. 2007; OECD 2006). The cress seeds (10) were placed in Petri container and exposed to 3 mL (undiluted, unfiltered) of sample for 72 h in incubator at 25 °C in dark medium. Then, Index of growth (IG %) was calculated by multiplying the germinated seed number exposed to sample (G1) and length of roots exposed to sample (L1) to be divided by the factor obtained from the multiplication of germinated seed number exposed to negative control medium (Gc) and length of root exposed to negative control medium (Lc). The experiments were performed triplicate. Negative control was performed with bottled water with very similar characteristics to feeding water (VERA, Italy).

2.5 Statistical Analysis

The EC_{50} values for *D. magna* test were calculated using PROBIT analysis. Windows Excel programme was used to calculate algae growth inhibition and *L. sativum* germination tests.

3 Results

3.1 Fluxes

Tables 1 and 2 show typical retanning bath procedures applied for production of clothing leathers (Nappa and wet blue respectively) in a large leather tannery district, Southern Italy, where more than 3000 types of chemicals are used in 140 tanneries (Meriç et al. 2005). The leather is processed in 4 phases: degreasing, chrome retanning, neutralization and re-tanning in which second syntan investigated in this study is used. According to Table 1, a total volume of 6 L of water is used for production of 1–2 kg raw pre-pickled leather (600% of water for 100% of leather) while 5 L water is used for the production of 1–2 of wet blue (Table 2). During these retanning procedures, syntans (ST1 and ST2) and resins are added to form softer leather at varying doses. For instance, 3% (30 g dissolved in 6 L of water) of ST2 and 4% (40 g in 6 L of water) of R1 are used in procedure one (Table 1) while 5% (50 g in 5 L of water) of ST2 is used together with other chemicals in the retanning process of wet blue leather (Table 2). Thus, a range of 2–4% for resins and 3–6% of syntans to be dissolved in an average value of 6 L of water are assumed to determine their fluxes as summarized in Table 3. As an example according to Tables 1 and 2, the concentration of the ST2 is assessed to vary from 5000 mg/l to 10000 mg/L in the retanning bath effluent. However, at least half of this compound remains on the leather product and less than half (<2500–5000 mg/L) is discharged in the effluent of the retanning bath which is connected to central treatment plant (Lofrano et al. 2007a). In the leather district approx. 1 ton of pre-pickled leather is processed a day which means around a total of 6000 m^3 /d of wastewater is originated from retanning baths while 2000 m^3/d of general wastewaters originates from floor washings, bathrooms and WC of the workers in the district which both flows gather a total of 8000 m^3/d of inflow of the central wastewater treatment plant as reported elsewhere (Meriç et al. 2005). Thereby the concentrations of the tanning chemicals will be diluted by 25% when they reach the central treatment plant. There has not been a reported evidence of the fate of these chemicals in activated treatment line, instead one textile syntan was reported as acute toxic on

Table 1 A retanning procedure applied in the leather-tanning district to process 1–2 kg of Nappa leather production

Operations	Percent for 100 g leather	Chemicals used	pH	Time (min)
Degreasing	150	H_2O 45°C		
	1.0	Ethoxylated nonylphenol (NPE)	3.8	40
	0.5	Ossalic acid		
Bolt Down and Washing	100	H_2O 40°C		
Chrome retanning	1	Phosphoric Oil		30
	0,15	Formic acid (85%)		10
	2	Chrome (IV)		12 h
	4	Synthetic chrome (R1)		
Bolt Down	200	H_2O 40°C		
Neutralization	2.0	Synthetic Salts	5.5	20
	0.8	Sodium bicarbonate		20
	1	Amino plastic resin		30
Bolt Down and Washing	150	H_2O 55°C		
Re-tanning	2	Plastic Resins	3.5	30
	4	Soya Lecithin		10
	3	Sulphonated oil		60
	1	Sulphonated oil		
	3	Vegetal oil		
	3	Syntan (ST2)		30
	3	Dye		
	3	Amino plastic resin		30
	3	Formic acid (85%)		40

activated sludge causing oxygen respiration inhibition, however, a 50–80% of removal due to biodegradation/absorption on the sludge flocs is assessed to calculate the effluent concentrations of the investigated chemicals. Furthermore, the effluent of the central wastewater treatment plant joins a municipal wastewater treatment plant with a total of 30000–35000 m^3/d inflow (De Feo et al. 2006) and flows a further activated sludge treatment in (diluted 1/4 and further 50–80% removal is assessed). The effluent of this joint treatment plant is discharged into the river flowing nearby which is the one of those most polluted rivers in EU and raising health concerns in summer months due to dry flow and subtraction for irrigation of its water (De Pippo et al. 2006).

According to above explained roughly assumptions the assessed fluxes of the investigated tannins and resins in the retanning bath effluent, central wastewater treatment plant influent and effluent and in the effluent of the joint municipal wastewater treatment plant are shown in Table 3.

3.2 Chemical Analyses

The ratio of BOD_5/COD was calculated very low compared to municipal wastewater for all compounds (Table 4). According to Fig. 1, the syntans cover the greater COD equivalents compared to the resins. However, the BOD_5/COD of RT1 and RT2 was also lower than syntans. Absorbance spectra for each compound were also different from each other, showing that the structures of syntans were more complex than resins (Fig. 2). The aromaticity of ST1 was bound to be the highest one, as well its double bound measure value (UV_{254}) was also the highest. However, UV measurements resulted in contrast to biodegradability (BOD_5/COD) which is generally found low related to high aromaticity and double bounds and acute toxic to activated sludge (Koyunluoglu et al. 2006) because ST2 corresponded lower UV_{254} and UV_{280} values.

On Table 4, a comparison for syntan data is given due to literature findings on a textile syntan (Koyunluoglu et al. 2006). Although COD and BOD values were different from the syntans investigated in

Table 2 A retanning procedure applied in the leather-tanning district to process 1–2 kg of wet blue leather production

Operations	Percent for 100 g leather	Chemicals used	pH	Time (min)
Degreasing	150			
	1.0	Etoxylated nonylphenol (NPE)	3.8	60
	0.5	Ossalic acid		
	0.5	EDTA		
Bolt Down and Washing	100			
Chrome retanning	2.0	Oil		20
	1	Sulphonated oil		
	0.3	Formic acid (85%)		15
	2	Chrome		30
	4	Synthetic chrome (R1)		
	3	Liquid syntan		13 h
Bolt Down	150			
Neutralization	2.0	Synthetic Salts	5.6	30
	0.6	Sodium bicarbonate		
	3	Resin		30
Bolt Down and Washing	100			
Re-tanning	3	Acrylic Resins (40%)	3	30
	5	Syntan (ST2)		60
	3	Syntan (ST1)		
	2	Resin (R2)		
	X	Dye		
	3	Sulfunated oil		50
	2	Sulphonated oil with sulphuric ester		
	2	Sulphonated oil		
	3	Resin stirol maelic		40
	X	Formic acid (85%)		60

X varies due to colour type

Table 3 The estimated fluxes of the investigated syntans and resins in the effluents

Concentrations (mg/L)	Percent used in 6 L of water	Re-tanning bath effluent[a]	Central Wastewater treatment plant inflow[b]	Central Wastewater treatment plant effluent[c]	Discharged to the river after mixed with the joint municipal wastewater treatment plant[d]
Syntans	3–6	2500–4167	1875–3125	937–1560[1] (375–625)[2]	117–195[1] (18–31)[2]
Resins	2–4	1667–3333	1250–2500	625–1250[1] (250–500)[2]	78–156[1] (12.5–25)[2]

[a] Based on Tables 1 and 2 and assumptions according to their annual consumption. 50% of the used amount is assessed to remain in the effluent

[b] Diluted by 25% due to other water fluxes from toilets, floor washing etc. reach the central wastewater treatment plant (A total volume of 6000 m^3/d originates from processes and 2000 m^3/d originates from general wastewater resources in the district)

[c] A range of 50([1])–80([2]) % removal/absorption on activated sludge is assumed, thus a range 20–50% is assessed to release in the central wastewater effluent

[d] A dilution ratio of 1/4 is assessed because the effluent of the District joins a municipal wastewater treatment plant nearby which has a total volume of 30,000–35,000 m^3/day inflow. A range of 50([1])–80([2]) % removal/absorption on activated sludge is assessed and 20–50% is assumed to release in the effluent to be discharged into the river flowing nearby.

Table 4 Physico-chemical characteristics of retanning chemicals studied

Compound	Description	COD (mg/L)	BOD_5 (mg/L)	UV_{254} 1/cm	UV_{280} 1/cm
ST1	Polycondensed of acids	755	30	18.02	13.95
ST2	Polycondensed of sulphonic acids	930	25	4.43	7.34
Textile Syntan	Koyunluoglu et al. (2006) (a methylene linked condensation product of arylsulphonic acids and hydroxyarylsulphone)	465 (155)[a]	6	13.74	11.78
R1	Retanning resin chromium based	135	25	3.28	1.67
R2	Retanning resin	215	12	0.21	0.13

[a] TOC Total organic carbon (mg/L)

this study, the structure of the ST1 seems to be similar with the literature one being a condensation of acids.

3.3 GC-MS Scanning

Because of their inherent properties (molar weight, Henrys constant etc.), tannins are difficult to be detected by gas chromatography. Various methods have been developed to detect vegetable tannins from plant extracts and in food and beverages and reversed-phase HPLC with UV-detection was most frequently used. For structural elucidations mass spectrometry and NMR-spectroscopy have been employed, but only a few articles deal with commercial vegetable tannin extracts used for leather production (Zywicki et al. 2002). Zywicki and co-workers (2002) developed reversed-phase HPLC–ESI–MS/MS methods to detect condensed and hydrolyzable tannins in tannery wastewaters by multiple reaction monitoring (MRM). The methods proved suitable even for highly loaded tannery wastewaters. So far no studies have dealt with the chromatograms of syntans used for leather production.

In Table 5 it is provided that chemicals determined after adoption GC-MS screening. Some of the compounds extracted (90% hits in GC library), confirmed previously reported findings (Lofrano et al. 2007b) where dibutyl phthalate and hexadecanoic acid were revealed in the GC-MS spectra of syntan solutions. Since derivatization procedure caused esterification of the fatty acids all fatty acids were determined as their respective methyl esters. Figures 3, 4, 5 and 6 show chromatograms for the studied compounds.

3.4 Toxicity

Positive control results for each biotest method used in this study are shown in Table 6, indicating that test results were in the range given in standard methods. The blank tests also resulted in acceptable for *D. magna* (<5% of immobilization and 1.4–1.8 mg/L of $K_2Cr_2O_7$ being in the range given in ISO method (1996)). The other positive control test results are given elsewhere (Guida et al. 2008) in accordance with literature findings.

ST2 displayed more sever toxicity than ST1 on *D. magna* (Fig. 7a). The same comparison is to be done for resins that R2 resulted in more toxic than R1 (Fig. 7b). However, both syntans were more toxic than resins. The calculated EC_{50} values for *D. magna* are shown in Table 5.

All chemicals investigated did not display any toxic effect on *A. salina* even at the highest concentrations tested (data not shown).

Algal growth inhibition tests confirmed the same trend with *D. magna*. According to Fig. 8, both syntans exhibited higher toxicity than resins, and syntans were more toxic than resins on *S. capricornutum*.

The test results with *L. sativum* showed that the growth of this species was affected by doses less than other species and this effect varied due to doses *vs*. chemicals (Fig. 9). The effect–dose relationship was linear (R^2 = 85–90) for the chemicals tested.

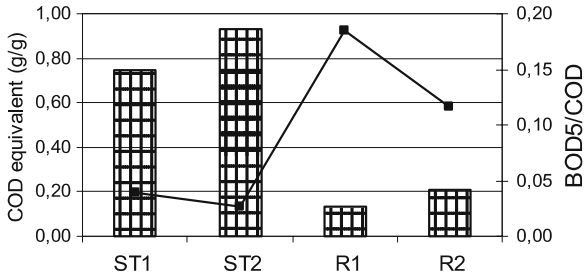

Fig. 1 COD equivalents *vs*. BOD_5/COD of retanning chemicals investigated

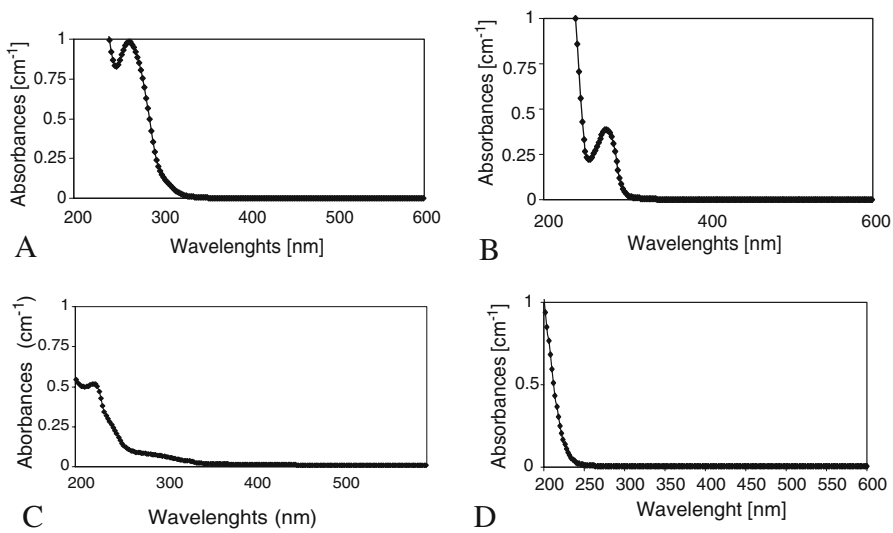

Fig. 2 UV–vis absorbance spectra for S1 (**a**), ST2 (**b**), R1 (**c**) and R2 (**d**)

According to estimated concentrations in different steps of the fateways of the investigated retanning chemicals they vary from 10 to 200 mg/L if they are trapped in the treatment line at 50 or 80%. If these ranges are compared with Table 6, *D. magna* is seen to be under risk after 48 exposure time to syntans according to its EC_{50} value. Our previous study on phenol based syntan also showed that *D. magna* was immobilization severely by 100–300 mg/l of syntan concentration (Lofrano et al. 2007b). The previous finding showed that sea urchins embryos and *D. tertilecta* growth were severely affected over 3 mg/L of the phenol based syntan (De Nicola et al. 2007b). If tannery effluent is discharged directly to the sea there can be foreseen very serious risk on these species while *A. salina* did not display any toxicity outcome according to this study results. This risk on sea urchin embryos and *D. tertiolecta* can be foreseen

Table 5 Tentative chemicals determined with derivatization in investigated compounds (>90% probability)

ST1	ST2	R1	R2
Trimethyl(2,6 ditert.-butylphenoxy) silane	Trimethyl(2,6 ditert.-butylphenoxy) silane	Trimethyl(2,6 ditert.-butylphenoxy)silane	Trimethyl(2,6 ditert-butylphenoxy)silane
Octanedioic acid	Octanedioic acid	Octanedioic acid	Octanedioic acid
Azelaic acid	Azelaic acid	Azelaic acid	Azelaic acid
Dibutyl phthalate	Dibutyl phthalate	Dibutyl phthalate	Dibutyl phthalate
Hexadecanoic acid	Hexadecanoic acid	Hexadecanoic acid	Hexadecanoic acid
Octadecanoic acid	Octadecanoic acid	Octadecanoic acid	Octadecanoic acid
Oleic Acid	Oleic acid	Oleic acid	Oleic acid
Trimethyl(4-(4-[(trimethylsilyl)oxy] benzyl)phenoxy)silane	Trimethyl(4-(4-[(trimethylsilyl)oxy] benzyl)phenoxy)silane	Heptanedioic acid	Heptanedioic acid
Hexadecanoic acid	Hexadecanoic acid	Dodecanoic acid	Nonanoic acid
4-Trimethylsiloxyphenylphenoxysulfone	2,2-Bis[(4-trimethylsiloxy)phenyl] propane	Cyclooctasiloxane, hexadecamethyl-	
Trimethyl(4-tert.-butylphenoxy)silane	Tetradecanoic acid	2,2′-Bis(trimethylsilyloxy) diphenylmethane	
4-Trimethylsiloxyphenyl-2′-trimethylsiloxyphenylsulfone		Binaphthyl sulfone	

S: synthetic tannin; R: resin

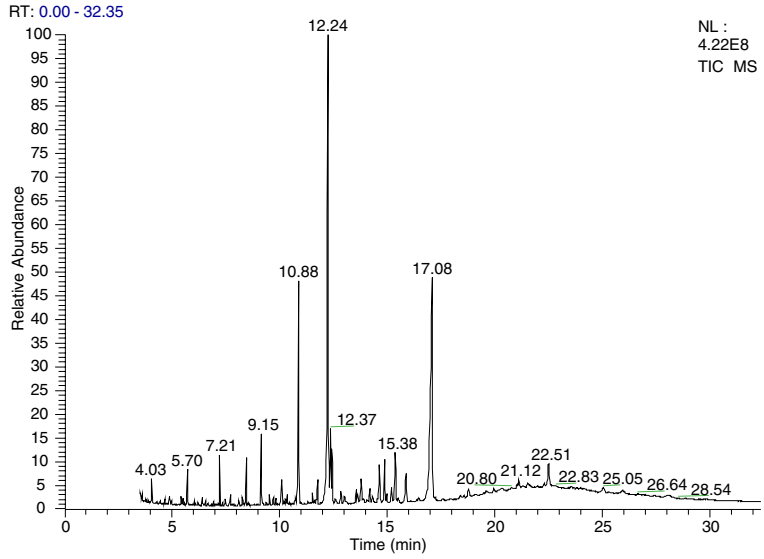

Fig. 3 GC-MS scanning for ST1 after LLE separation and derivatization

even for the effluent pathway as in this study, if a 100% dilution is expected along the river basin. Because these are roughly estimated concentrations can vary from 50 to 100% even due to seasonal changes, peaks use, treatment plants conditions these range can be expected to increase by two-fold. However, their fate pathways in the river water are completely unknown due to lacking literature such as sinks, photodegradation, biodegradation and bioaccumulation. Because the district treatment plant has an equalization tank with 1-day volume no daily peaks are expected to occur, however, according to leather product type, the chemicals used in the process are also changed and this raises the concern of interactions among the chemicals used. Therefore, the best way of explaining whole assumptions done hereby is to investigate the fate of these chemicals in real wastewater samples.

4 Discussion

As an obvious result, the bioassays used resulted in different sensitivity and end-points. As reported in an

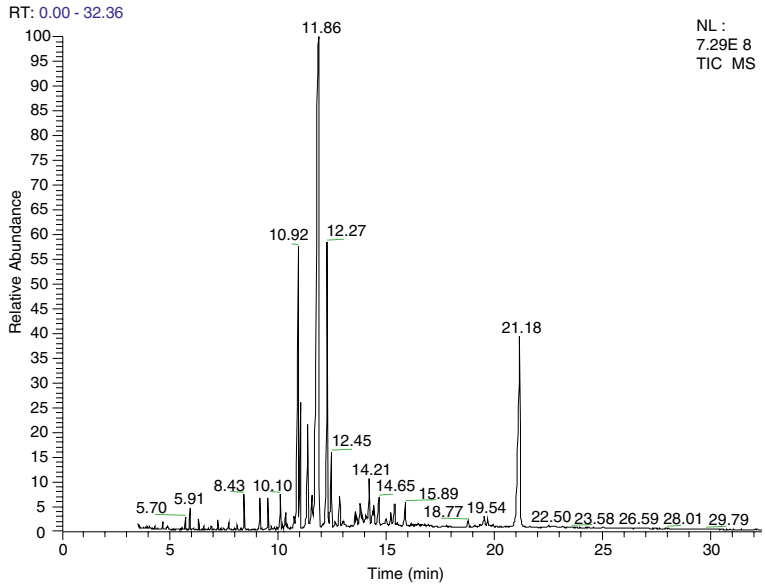

Fig. 4 GC-MS scanning for ST2 after LLE separation and derivatization

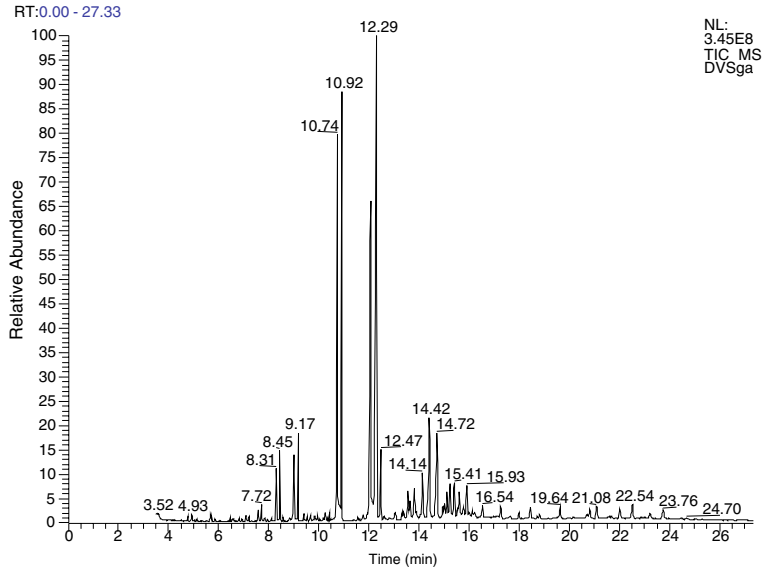

Fig. 5 GC-MS scanning for R1 after LLE separation and derivatization

interlaboratory calibration study (Farré et al. 2007), toxicity tests using different species on wastewater effluents were found at different sensitivities and endpoints, and only a limited number of tests were standardized and validated (Farré et al. 2007). This was the case also for the leather tannery wastewater surveys (Oral et al. 2007; De Nicola et al. 2007a). For the tested compounds *D. magna* and *S. capricornutum* were found to be the most sensitive species.

There was an evident correlative tendency between COD (BOD_5/COD ratio also) and toxicity results. As seen in Fig. 1 and Table 2, ST2 corresponds to the highest COD and the lowest BOD_5 /COD ratio as well as the most toxic one on *D. magna* and *S. capricornutum* (Figs. 7 and 8). The similar comment can be added for both syntans, being more toxic than resins, that they are corresponding higher COD values and lower BOD_5/COD ratios than resins. On the contrary of biodegradability evaluation, UV–VIS did not seem to be related to toxicity of the tested syntans, however, confirmed that the compounds with higher UV–vis values result in more toxic (syntans > resins).

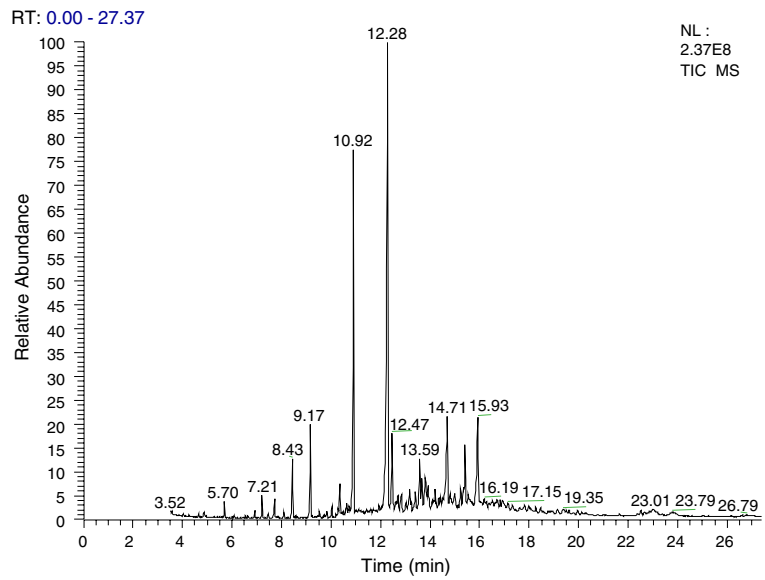

Fig. 6 GC-MS scanning for R2 after LLE separation and derivatization

Table 6 EC_{50} values for *Daphnia magna* calculated by PROBIT analysis

Exposure time (h)	Toxicity	ST1	ST2	R1	R2
24	EC_{50} (mg/L)	556	364	>1000	766
	(95% confidence limit)	(448–702)	(NA)	(NA)	(611–1072)
48	EC_{50} (mg/L)	129	NA	974	419
	(95% confidence limit)	(59–173)	(NA)	(861–1286)	(326–529)

NA not available: data do not fit PROBIT profile.

A. salina was observed not sensitive for the tested compounds although it was used for leather tannery wastewater testing as reliable one (Sauer et al. 2006).

Test results of *L. sativum* as calculated on the basis of an integrated index did not show any significant distinction among the compounds. This was the case also for each of the based end points individually (data not shown). This may be due to the characteristic of this specie or, the compounds even at the highest concentration were not effectively assimilated by the plant cells although this specie was found very effective to test very complex mixtures, in particular the metals as free or bound in municipal wastewater sludge (Fuentes et al. 2006).

Chromatographic scanning provides highly appreciated information for the identification chemical composition of complex chemicals, in particular for the mixture compounds to validate also with toxicity tests (Hernando et al. 2005). Beyond choosing the proper techniques/instruments the extraction, derivatization methods are up-to-date to be optimized according to mixture structures.

No correlation was observed between toxicity and GC-MS data. For instance, there has been high number of compounds scanned in R1 solution while it was less toxic on *D. magna* and S. *capricornutum* among all tested compounds. This conflict may be due to undefined other chemicals in other investigated chemicals in this study which needs further determinations.

Leather tannery is well known with its high salinity (from 2000 to 8000 mg/L) (Kabdasli et al. 1993), thus it is expected to use more saline water species to test these effluents (Meriç et al. 2005), or comparative testing procedures (Oral et al. 2007 or De Nicola et al. 2007a). Keeping in mind that in our previous findings there was an accordance with the tannins (De Nicola et al. 2007b) and real wastewater (Meriç et al. 2005) tested for the toxicity by marine organisms, here the

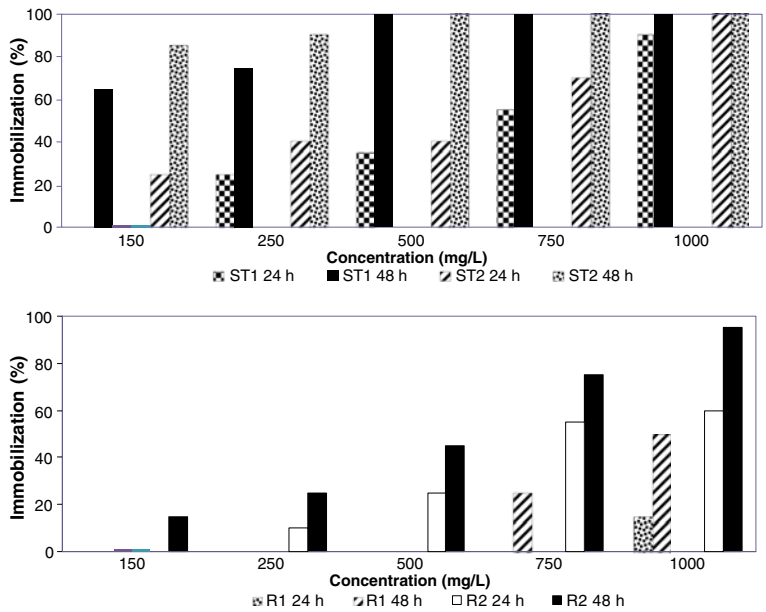

Fig. 7 Immobilization of *Daphnia magna* exposed to synthetic tannins (**a**) and resins (**b**) (Quadruplicate experiments for 24 and 48 exposure time)

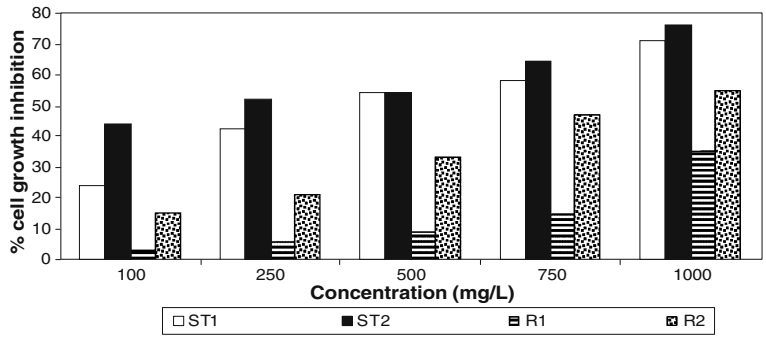

Fig. 8 Cell growth inhibition of *Selenastrum capricornutum* exposed to synthetic tannins (ST1, ST2) and resins (R1, R2) (96 h, triplicate experiments)

results showing the sensitivity of freshwater species to tanning materials raise the question of "*proposing the right test battery to assess the risk in tannery effluents?*"

5 Conclusion

This study attempted to evaluate source-flux-structure-biodegradability-toxicity characteristics of four retanning agents as being multi-approach and the first one in these aspects, in particular for the tested species and GC-MS scanning. The main outlines of the study are to be summarized as the following:

- The toxicity tests showed different end-comes. *D. magna* and *S. capricornutum* as freshwater organisms were more sensitive to tested products than *A. salina* which did not result in any toxic effect or *L. sativum* which was also not very much indicative to distinguish the toxic severity among the tested compounds. *S. capricornutum* was more sensitive than *D. magna* as expected result. Both these species indicated that syntans were more toxic than resins. However, both fresh water and marine organisms are proposed to use for impact assessment of tannery chemicals and in tannery real effluents to have exact fate pathway of these chemicals in treatment lines and in discharged aquatic environment.
- Indeed, the flux evaluation based on rough assumptions for the pathways of the investigated compounds raised risk factor for the bio tests used in this study.
- Biodegradability (BOD_5/COD ratio) was a significant parameter to indicate also the toxic effects of the compounds studied on *D. magna* and *S. capricornutum* among tested species.
- UV–vis was not a direct parameter to explain the trend in toxicity outcomes, however confirmed that the compounds with more complex structure (high UV–vis values) resulted in more toxic.
- GC-MS scanning results were not correlated with toxicity outcomes. This is attributed to the extraction and derivations procedures to be improved to define other chemicals, which can cause toxicity in synthetic tannins.
- A detailed work is needed to simulate the results of this study in the tannery wastewater.

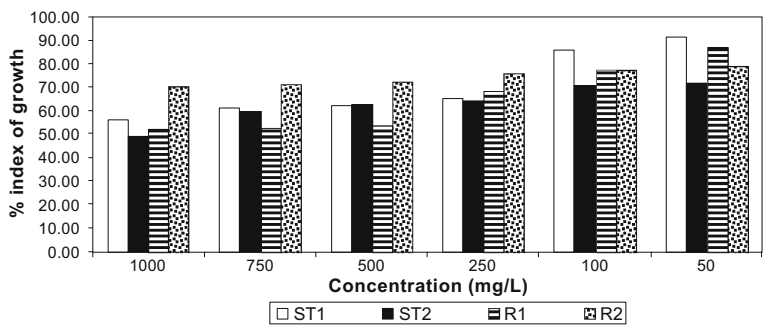

Fig. 9 Inhibition of growth of *Lepidium sativum* exposed to retanning chemicals investigated (72 h, triplicate experiments)

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